REMARKS

I. Status of the Application:

Claims 1-69 are pending in the application. Claims 43, 45-51, and 53-57 have been allowed. (Office action, page 1.) Applicants note that the Office has determined that claims 12, 15-28, 40, and 43-69 are free of the prior art, which the Office admits does not teach the use of *Renilla* luciferase in yeast and does not teach the use of low copy number plasmids in a yeast two-hybrid assay. (*Id.*, pages 9 and 10.)

The Office has raised several objections to the specification. (Office action, pages 2 and 3.) First, the Office objects to the specification for failing to comply with the requirements of 37 C.F.R. § 1.821. (Office action, page 2.) According to the Raw Sequence Listing Error Report, the diskette containing the computer readable form ("CRF") of the sequence listing, which Applicants submitted on April 13, 2001, may contain a bad disc sector. Applicants enclose a substitute CRF of the sequence listing.[1] The statement required by 37 C.F.R. § 1.825(d) has been submitted on this date in a separate paper.

Second, the Office notes that the specification contains sequences without the appropriate sequence identifier numbers. (Office action, page 2.) Applicants have amended the specification to recite these numbers.

Third, the Office requires Applicants to correct the Brief Description of the Drawings. (*Id.*, page 3.) Applicants have made the requested amendments.

[1] The Office states that "Applicant[s] must provide a paper copy [of the sequence listing,]" however, there is no indication that the paper copy submitted on April 13, 2001, fails in any way to comply with the requirements of 37 C.F.R. §§ 1.821-1.825. Accordingly, Applicants are not submitting a substitute paper copy with this Amendment.

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In view of these amendments and remarks, Applicants request withdrawal of each of the objections to the specification.

Claims 7, 23, 39, and 64 have been objected to because of certain informalities. (*Id.*, page 3.) Applicants submit that these objections are moot in view of the amendments made herein. Thus, they request reconsideration and withdrawal of the objections.

Applicants have amended claims 7, 23, 29, 39, 43, 44, 52, 58, and 64 solely to more clearly recite their invention. Support for these amendments is found in the specification and claims as originally filed. No new matter has been entered.

Finally, Applicants have amended the title for Example 10 to correct a typographical error.

II. The Claims Comply With 35 U.S.C. § 112, Second Paragraph

Claims 29-42, 44, 52, and 58-69 have been rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter Applicants regard as their invention. (Office action, page 4.) The Office specifically addresses language appearing in claims 29, 32, 44, 52, 58, and 59. Applicants traverse the rejections.

For claim 29, the Office contends that the claim is indefinite because "it contains a step of incubating a test sample with the yeast cell but the method does not appear to involve test samples." (*Id.*) The Office also asserts that the claim is incomplete because "it does not appear to contain a positive process step which clearly relates back to the preamble as the last recited step is the detection of luciferase gene

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expression but the method is drawn to peptide interactions." (*Id.*) Claim 58 is rejected on a similar basis. (*Id.*, page 5.)

Applicants have amended claims 29 and 58 solely to more clearly recite their invention. Although they do not believe that the rejection of these claims is proper, in view of the amendments made herein Applicants submit that the rejections are now moot. They request withdrawal of the rejections.

Regarding claim 32, the Office asserts that it is "indefinite as it is drawn to detecting the presence or absence of luciferase activity in the first and second yeast cells in step (iv) and then looking for a change in luciferase activity in step (v)." (Office action, page 4.) According to the Office, "[i]t is unclear how the change in activity in step (v) relates to the presence or absence of activity in step (iv)." (*Id.*)

Claim 32, however, does not recite steps (iv) and (v). The undersigned telephoned Examiner Schwartzman in August 2001 asking for clarification. During a conversation on August 27, 2001, the Examiner informed that undersigned that a typographical error had been made and the rejection applies to claim 43. Applicants traverse the rejection as applied to claim 43.

While they traverse the rejection, Applicants have amended claim 43 to more clearly recite their invention. The claim, in particular part (v), has been amended to track the language of claim 48 in parent U.S. Patent No. 5,989,808. Claim 43, as amended, recites that detection of lower luciferase activity in one of the yeast cells compared to the other yeast cell provides the indication that the test sample binds to the heterogeneous fusion protein encoded by the nucleotide sequence present at a copy number of 1 or 2 in that yeast cell exhibiting lower luciferase activity, thereby affecting

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the binding interaction of the peptide binding pair. Applicants submit that this amendment addresses the Office's concern regarding the clarity of the claim and, therefore, withdrawal of this rejection is appropriate.

Claims 44 and 52 have been rejected because they are allegedly "vague and indefinite as it is unclear which yeast cell is being referred to since parent claim 43 is drawn to two different yeast cells." (Office action, page 5.) Applicants traverse the rejection as moot because the amendment of these claims clarifies which yeast cell of the two cells recited in parent claim 43 is being referred to.

Claim 59 has been rejected because "it is unclear which yeast cell is being referred to since parent claim 58 is drawn to two different yeast cells." (Office action, page 5.) Claim 58, however, does not recite two different yeast cells. Accordingly, the Office should withdraw the rejection.

In view of these amendments and remarks, Applicants request reconsideration and withdrawal of each of the rejections under section 112, second paragraph.

III. The Claims Are Patentable Over the Cited References

The Office has rejected claims 1-11, 13, 14, 29-39, 41, and 42 under 35 U.S.C. § 103(a) as allegedly unpatentable over U.S. Patent No. 5,283,173 to Fields *et al.* ("Fields") in view of any one of U.S. Patent Nos. 5,525,490 to Erickson *et al.* ("Erickson"), 5,834,209 to Korsmeyer ("Korsmeyer"), or 5,837,478 to Gallatin *et al.* ("Gallatin"), and further in view of U.S. Patent No. 5,641,641 to Wood ("Wood"). (Office action, page 6.) Applicants traverse this rejection.

Fields is cited as teaching a yeast two-hybrid system in which the first peptide of a peptide binding pair is fused to a DNA binding domain and a transcriptional activation

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protein and a second peptide of the binding pair is fused to an activation domain of the transcriptional activation protein. (*Id.*) Erickson, Korsmeyer, and Gallatin are cited as teaching the use of luciferase as a reporter gene in a two-hybrid assay. (*Id.*, page 7.) Wood is cited as providing detailed guidance on the use of luciferase, particularly *Photinus* luciferase, "as a reporter in many cell types, including yeast." (*Id.*)

With respect to the asserted teachings of Korsmeyer and Gallatin, Applicants point out that the specific disclosure the Office relies on in these references is not prior art to the rejected claims. The use of luciferase as a reporter gene is disclosed in application no. 08/259,609, filed on June 14, 1994, to which Applicants have requested benefit under 35 U.S.C. § 120. Korsmeyer and Gallatin are each based on a series of continuation-in-part applications, which have U.S. priority dates earlier than June 1994. The relevant disclosure, however, was not added until after June 1994. For example, U.S. Patent No. 5,470,953 to Gallatin *et al.* issued from a continuation-in-part application filed August 5, 1994, that does not contain the disclosure concerning luciferase in col. 7, lines 39-67, cited by the Office. This disclosure must have been added in one of the three later CIPs that culminated in Gallatin.

Similarly, U.S. Patent No. 5,622,852 to Korsmeyer, which issued from a CIP filed October 31, 1994, is the first application in the chain culminating in the reference patent containing the cited disclosure concerning luciferase found at col. 42, lines 17-41. U.S. Patent No. 5,700,638 to Korsmeyer, which issued from an application filed May 25, 1994, does not contain this disclosure. Accordingly, it is inappropriate for the Office to rely on the cited teachings of Korsmeyer or Gallatin in making the instant rejection.

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Given the unavailability of Gallatin and Korsmeyer as prior art, the rejection reduces to Fields in view of Erickson and further in view of Woods. Erickson, however, was cited by the Office during prosecution of related U.S. Patent No. 5,989,808. Applicants obviated rejections based on Erickson because in Erickson's assay the reporter gene is *indirectly* and *negatively* regulated by the reconstituted transcriptional activation protein, via the product of a relay gene.

In the instant invention, activation of the reporter gene is *directly* and *positively* regulated by the reconstituted transcriptional activation protein. In the absence of this protein, there is no expression of the reporter gene. When a reconstituted transcription activation protein is present, (as occurs when a peptide binding pair is formed), the reporter gene is expressed. This is evident from the claim language reciting "a reporter gene activated under positive transcriptional control of the reconstituted transcriptional activation protein." The direct, positive transcriptional control of Applicants' reporter gene means that no relay gene is required. The reporter gene in Erickson is controlled in a manner exactly opposite to that recited in the rejected claims.

In Erickson's system, the reporter gene is *indirectly* and *negatively* regulated by the reconstituted transcriptional activation protein, via the product of the relay gene. When a reconstituted transcriptional activation protein is present in Erickson's reverse system, the relay gene is expressed and its product functions to repress transcription of the reporter gene. On the other hand, when the reconstituted transcriptional activation protein is absent the relay gene is not expressed. In the absence of the repressor, the reporter gene is activated, that is, expressed. (*Id.*, lines 49-57.)

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Erickson also does not teach or suggest the use of luciferase as a reporter gene in a two-hybrid assay as recited in the instant claims. Erickson only teaches a reverse two- hybrid assay that is clearly different than the claimed assays.

Furthermore, Erickson expressly teaches away from the claimed invention. According to Erickson, "two-hybrid systems that produce a positive readout contingent upon intermolecular binding of the two hybrid proteins are generally not suitable for screening for agents which inhibit binding of the two hybrid proteins," (Erickson, column 2, lines 53-56), and "[p]resent two-hybrid methods" relying on a positive readout do not afford a method for identifying binding inhibitors or competitors with satisfactory sensitivity or selectivity. (*Id.*, lines 62-65; emphasis added.) Rather, Erickson teaches that a "suitable" assay requires his reverse two-hybrid system. Thus, Erickson expressly teaches away from the claimed invention. That a prior art reference teaches away from a claimed invention is a significant factor to be considered when determining obviousness. M.P.E.P. § 2145.

In view of Erickson's teaching, Applicants submit that the claims are not *prima* facie obvious over the combination of Fields, Erickson, and Wood. Fields is cited as teaching a two-hybrid assay, but not as teaching the use of luciferase as a reporter. (Office action, page 7.) Erickson teaches a reverse two-hybrid assay that is clearly different than Applicants. This reference expressly teaches away from the claimed invention. Given the teaching of Erickson, Applicants submit that one of ordinary skill in the art would not have been motivated to combine the teaching of Fields and Erickson to arrive at the claimed invention.

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Adding the teaching of Wood does not cure the problems evident in the combination of Fields and Erickson because Wood does not teach or suggest two-hybrid assays. In fact, the Office has mischaracterized the teaching of Wood. Wood does not describe the use of luciferase "as a reporter gene in many cell types, including yeast." (Office action, page 7.) Rather, Wood describes the use of luciferase as a reporter in assays involving extracts prepared from yeast cells. This is evident in Wood's description at col. 8, line 61 to col. 9, line 46, of the types of sample that can be assayed for the presence of luciferase and in Examples 1 and 2.

Applicants therefore submit that the Office has not established a *prima facie* case of obviousness of claims 1-11, 13, 14, 29-39, 41, and 42 over the cited references. Accordingly, Applicants request reconsideration and withdrawal of this rejection.

IV. Rejection of Claims 1-28 for Obviousness-Type Double Patenting

Claims 1-28 stand provisionally rejected under the doctrine of obviousness-type double patenting over claims 49-136 of copending application no. 09/305,483. (Office action, page 9.) Applicants traverse this rejection, however, they respectfully request that this issue be held in abeyance until allowable subject matter is indicated.

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CONCLUSION

In view of the above amendments and remarks, Applicants submit that this application is in condition for allowance. An early and favorable response from the Office is earnestly solicited.

Please grant any extensions of time required to enter this Amendment and charge any additional required fees to our deposit account 06-0916.

Respectfully submitted,

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By: _____
Steven P. O'Connor
Reg. No. 41,225

Dated: January 4, 2002

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APPENDIX I
Specification Amendments

Replace the first paragraph on page 1 with the following paragraph:

This application is a continuation-in-part of application Serial No. 09/305,483, filed May 9, 1999, now U.S. Patent No. 6,284,519, which is a continuation of application Serial No. 08/259,609, filed June 14, 1994, now U.S. Patent No. 5,989,808, which are incorporated by reference herein.

Replace the second paragraph on page 9 with the following paragraph:

[Figure 2 contains] Figures 2A and 2B are photographs of plates which show the results of the growth experiments conducted in Example 1 for stains CY722, CY723, CY724, and CY781 on non-selective medium and selective medium, photographs A and B, respectively.

Replace the paragraph bridging pages 25 and 26 with the following paragraph:

Oligonucleotides prepared on an ABI oligosynthesizer are designed according to the published cDNA sequence for pig GH (*see* Su and El-Gewely, 1988). A 30 base 5' oligonucleotide contains a NcoI site (5'-CATGCCATGGAGGCCTTCCCAGCCATGCCC 3') (SEQ ID NO: 1) and a 27 base 3' oligonucleotide contains a BamHI site (5'-CGGGATCCGCAACTAGAAGGCACAGCT-3') (SEQ ID NO: 2). The GH cDNA is generated using a pig pituitary lambda gt11 library as template source. A 540 bp fragment is obtained, ligated into pCR II vector (Invitrogen Corp.), recombinants are confirmed by restriction enzyme digest, and the DNA produced as described in Maniatus *et al.*, 1982. The cDNA sequence is confirmed by di-deoxy terminator reaction using reagents and protocols from Perkin-Elmer Cetus Corp. and an ABI 373A automated sequencer. The GH cDNA is directionally cloned into pACT-II via NcoI and

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BamHI sites. The cDNA encoding the extracellular domain of the GHR is generated using standard PCR methods. A 33 base 5' oligonucleotide containing a NcoI site (5'-CATGCCATGGAGATGTTTCCTGGAAGTGGGGCT-3') (SEQ ID NO: 3) and a 39 base 3' oligonucleotide containing a termination codon, followed by a NcoI site (5'-CATGCCATGGCCTACCGGAAATCTTCTTCACATGCTGCC-3') (SEQ ID NO: 4) are used to generate a 742 bp fragment encoding amino acids 1-**247** of the rat GHR (Baumbach *et al.*, 1989). This GHR cDNA is cloned into vector pCRII as previously described above, and then subcloned into the NcoI site of vector pAS2. DNA of the final recombinant vectors is transformed into yeast strain(s) by the lithium acetate method (Rose *et al.*, 1990).

Replace the paragraph bridging pages 27 and 28 with the following paragraph:

To substantiate the apparent binding of GH to its receptor in the foreign environment of a yeast nucleus, the system is modified to add a third plasmid mediating expression of "free" ligand to show that the GH peptide competes with the GH-Gal4 fusion protein, reversing the 2-hybrid interaction shown in Example 1. The parental strain Y190 (Wade Harper *et al.*, 1993) is grown on a medium containing 5-fluoro-orotic acid to select for derivatives that spontaneously lose the URA3 gene (see Rose *et al.*, 1990). The resultant strain, designated CY770, is utilized for all experiments examining the effects of protein expressed concurrently from the third component, (that is, the third plasmid). The cDNA encoding GH is generated by PCR methods using a 38 base 5' oligonucleotide containing an EcoRI site (5'-CCGAATTCAAAATGGCCTTCCCAGCCATGCCCTTGTCC-3') (SEQ ID NO: 5) and a 26 base 3' oligonucleotide containing a HindIII site

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(5'CCAAGCTTCAACTAGAAGGCACAGCT-3') (SEQ ID NO: 6) for subsequent subcloning into the vector pCUP. pCUP is an inducible yeast expression vector derived from pRS316 (Hill *et al.*, 1986). Briefly, this vector is constructed by inserting the 3' end of the yeast PGK gene (from pPGK; Kang *et al.*, 1990) into the pRS316 cloning region as a BamHI-SalI fragment to serve as a transcriptional terminator. To this plasmid, the CUP1 promoter region (Butt *et al.*, 1984) is amplified by PCR as a SacI-EcoRI fragment and inserted into corresponding sites of the plasmid to create pCUP. The GH expression plasmid (GH-pCUP) is then co-transformed with the GH and GHR fusion constructs into strain CY770 to generate CY781. Concurrent expression of free GH with the GH and GHR fusion proteins (CY781) is shown to block GH-GHR-dependent cell growth on selective medium (Figure 2B). This experiment typifies an *in vivo* competition assay and demonstrates the reversibility of the observed ligand-receptor interaction.

Replace the first paragraph on page 29 with the following paragraph:

To expand and validate this technology, a similar system was developed using the peptide hormone prolactin (PRL) and its receptor. Prolactin is structurally related to GH and the prolactin receptor (PRLR) is also a member of the cytokine receptor superfamily. Unlike human GH, sub-primate GH does not readily bind the PRLR (Young and Bazer, 1989); nor does PRL readily bind the GHR (Leung *et al.*, 1987). Mature porcine PRL is generated as a fusion to the GAL4 activation domain. Oligonucleotides are designed to pig PRL (obtained from GenBank; Accessign No. X14068), and used to generate the mature pig PRL protein hormone from a pig pituitary lambda gt11 library, using standard PCR methods. A 31 base 5' oligonucleotide

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includes an EcoRI site (5'-CGGAATTCTGCCCATCTGCCCCAGCGGGCCT-3') (SEQ ID NO: 7) and corresponds to sequences encoding amino acids 1-7. A 30 base 3' oligonucleotide contains an EcoRI site (5'-GAATTCACGTGGGCTTAGCAGTTGCTGTCG-3') (SEQ ID NO: 8) and corresponds to a region of cDNA 3' to the endogenous termination codon. A 600 bp fragment is obtained, ligated into vector pCR II, and confirmed by restriction enzyme digest and sequence analysis. The PRL cDNA is cloned into pACT-II via the EcoRI site.

Replace the paragraph bridging pages 29 and 30 with the following paragraph:

The extracellular domain of the porcine PRL receptor (PRLR) is generated as a fusion to GAL4 DNA binding domain. Oligonucleotides are designed based on sequence of the mouse PRLR (Davis and Linzer, 1989) A 31 base 5' oligonucleotide contains a SmaI site (5'-TCCCCCGGGGATGTCATCTGCACTTGCTTAC-3') (SEQ ID NO: 9) while the 31 base 3' oligonucleotide contains a termination codon followed by a SalI site (5'TCCGTCGACGGTCTTTCAAGGTGAAGTCATT-3') (SEQ ID NO: 10). These oligonucleotides flank the extracellular domain of the PRLR, encoding amino acids 1-229. A pig pituitary lambda gt11 library is used as a template source. Using standard PCR methods, a 687 bp fragment is generated, ligated into pCRII, and the nucleotide sequence is confirmed. The PRLR cDNA is cloned into the pAS2 vector via the SmaI and SalI restriction sites.

Replace the first paragraph on page 31 with the following paragraph:

Additional strains are developed to assess ligand-receptor specificity. URA- strains expressing GH and GHR fusion proteins are transformed with pCUP or PRL-pCUP, while strains expressing PRL and PRLR fusion proteins are transformed with

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pCUP, or PRL-pCUP. Briefly, PRL-pCUP is constructed in a fashion similar to that described for GH-pCUP. The PRL cDNA is generated by PCR using a 33 base 5' oligonucleotide with an EcoRI site (5'-GAATTCAAAATGCTGCCCATCTGCCCCAGCGGG-3') (SEQ ID NO: 11) and the 3' oligonucleotide in example 1B. The resulting fragment is introduced into pCUP via the EcoRI site. As demonstrated in the above Examples, a strain expressing the GH and GHR fusions with no competitor grows on selective medium and this growth is abolished with coexpression of free GH. The prolactin experiment produces similar results, which confirms the specificity of the ligand-receptor binding in the yeast cell. A strain carrying PRL and PRLR fusions (CY787) can grow on selective medium and this growth is abrogated by expression of free PRL (CY786; Table 1).

Replace the paragraph bridging pages 33 and 34 with the following paragraph:

Low-copy-number plasmids expressing GHR- or GH-Gal4 fusion proteins (pOZ153 and pOZ152, respectively) are constructed to reduce expression of these proteins. In addition, a novel reporter gene is constructed that prevents cell proliferation on selective medium unless expression is abrogated. To construct the GHR fusion expression plasmid, a SacI-BamHI restriction fragment containing a yeast constitutive promoter and GAL4 sequences is isolated from pAS1 (Durfee *et al.*, 1993) and cloned into pUN30 (Elledge and Davis, 1988). The extracellular domain of GHR is then fused to GAL4 by ligation as an NcoI fragment as described in Example 1 to create pOZ153. To construct the GH fusion expression construct the entire GH-Gal4 region with promoter and terminator sequences is isolated from the plasmid described in Example 1 as a PvuI-SalI fragment. This DNA segment is cloned into pUN100 (Elledge and Davis,

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1988) generating pOZ152. A reporter gene is constructed by isolating the yeast CYH2 coding region and operatively linking it to a GAL promoter in a yeast expression plasmid. Briefly, the GAL1 promoter region is inserted into YEp352 (Hill *et al.*, 1986) as a 685 bp EcoRI-BamHI fragment. CYH2 sequences are amplified by PCR using oligonucleotides primers (5'-GGATCCAATCAAGAATGCCTTCCAGAT-3' (SEQ ID NO: 12) and 5'-GCATGCGTCATAGAAATAATACAG-3' (SEQ ID NO: 13)) and pAS2 as the template. The PCR product is digested with BamHI plus SphI and cloned into the corresponding sites in the YEp352-GAL vector. These plasmids are transformed into yeast strain CY770 which carries a mutation at the chromosomal cyh2 gene rendering the strain resistant to the protein synthesis inhibitor cycloheximide. The presence of all three plasmids is necessary to confer cycloheximide sensitivity (cyh^s).

Replace the paragraph bridging pages 36 and 37 with the following paragraph:

The interaction of vascular endothelial cell growth factor (VEGF) with the ligand binding domain of its cognate receptor (KDR, kinase insert domain containing receptor) is described as an example for this system. KDR is a tyrosine kinase receptor, and dimer formation (1 ligand - 2 receptors) is suggested to be important for hormone-induced receptor function. The cDNA encoding the ligand domain of KDR (Terman *et al.*, 1991) is isolated as an NCO I - BamHI fragment and cloned into both the pACT-II and pAS2 vectors. The cDNA encoding the mature protein for VEGF is generated using standard PCR techniques. Oligonucleotides are designed from published sequence (see Fischer *et al.*, 1991). A 34 base 5' oligonucleotide containing an EcoRI site (5'-CGGAATTCGAAGTATGGCACCCATGGCAGAAGGA-3') (SEQ ID NO: 14) and a 28 base 3' oligonucleotide containing an EcoRI site (5'-

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CGGAATTCGGATCCTCATTCATTCATCA-3') (SEQ ID NO: 15) are used to generate a 450 bp fragment encoding the mature protein and cloned into the EcoRI site of pCUP. DNA of final recombinant vectors is transformed into yeast by the lithium acetate method to generate appropriate strains.

Replace the paragraph bridging pages 39 and 40 with the following paragraph:

The luciferase reporter plasmid(s) for use in yeast two-hybrid applications were generated as follows. Plasmid pEK1 (described in Price et al., 1995) was digested with BamHI+SalI restriction enzymes, dephosphorylated, and gel purified. The luciferase coding region was obtained as a Bgl II – Sal I 1.9 kb cDNA fragment from pGL3Basic (Promega), purified, and ligated to the prepared pEK1 vector using standard procedures to generated plasmid Kp126. The recombinant DNA is transformed into DH5α *E. coli* using standard procedures and plasmid DNA prepared. The construct was confirmed by restriction enzyme analysis and cDNA sequence analysis (using primer Kx38: 5'-TCAAATTAACAACCATAGGAT-3') (SEQ ID NO: 16). Kp126 was used extensively for yeast two-hybrid systems and retains the original mammalian Kozak sequence from original DNA source.

Replace the first full paragraph on page 43 with the following paragraph:

The complete ORFs for G-alphaZ and RGS-Z were isolated from a human brain cDNA library (Quickclone cDNA, Clontech) by PCR amplification. PCR primers were designed 5' and 3' of the open reading frame of G-alphaZ (GenBank#J03260) and RGS-Z (Genbank#AF074979). PCR amplification was performed under standard buffer conditions using the Clontech cDNA Advantage cDNA kit. The primers were G-alphaZ-fwd 5' ACCATGGGATGTCGGCAAAGCTCAGAGGAAA-3' (SEQ ID NO: 17) and G-

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alphaZ-rev 5'-CAAGGGGTGGGGGACATT-3' (SEQ ID NO: 18) for G-alphaZ and RGS-Z-fwd 5'-CCCGGCCGGCAGGTGGAC-3' (SEQ ID NO: 19) and RGS-Z-rev 5'-CTCATGCAAAATAAAAGTGGTTC-3' (SEQ ID NO: 20) for RGS-Z. Cycle parameters were 94°C for 30 seconds, 55°C for 30 seconds and 72°C for 1 minute for a total of 30 cycles.

Replace the second full paragraph on page 44 with the following paragraph:

A constitutively active (Q205L; Wang et al., 1998) form of G-alphaZ was generated. The Q205L mutation in G-alphaZ was generated in the GaZ-pGBKT7 and GaZ-pACT2 plasmids using the QuickChange kit (Stratagene) and the primers 5'-GTGGGGGGGCTGAGGTCAGAG-3' (SEQ ID NO: 21) and 5'-CTCTGACCTCAGCCCCCCCAC-3' (SEQ ID NO: 22). Recombinant mutant plasmids were transformed into bacterial cells and DNA isolated using standard methods. The Q205L G-alphaZ mutants were identified by sequence analysis of the resulting colonies and termed Q205L/GaZ-pGBKT7 and Q205L/GaZ-pACT2.

On page 54, replace the heading for Example 10 with the following:

Example 10: [Multiplexes] Multiplex assays using strains expressing different luciferase reporter genes (Firefly Luc reporters and Renilla Luc reporter)

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APPENDIX II
Claim Amendments

7. (Amended) The yeast cell of claim 6 wherein the peptide is a growth factor selected from the group consisting of epidermal growth factor, nerve growth factor, leukemia inhibitory factor, fibroblast growth factor, platelet-derived growth factor, vascular endothelial growth factor, tumor necrosis factor, oncostatin M, ciliary neurotrophic factor, erythropoietin, steel factor, placental lactogen, and [TGF] transforming growth factor β ("TGF").

23. (Amended) The yeast cell of claim [15] 22 wherein the DNA binding protein is selected from the group consisting of a mammalian steroid receptor and bacterial LexA protein.

29. (Amended) A method of detecting the interaction of a first peptide and a second peptide of a peptide binding pair in the presence of a test sample, comprising:

(i) culturing at least one yeast cell, wherein the yeast cell comprises;

a) a nucleotide sequence encoding a first heterologous fusion protein comprising the first peptide or a segment thereof joined to a transcriptional activation protein DNA binding domain;

b) a nucleotide sequence encoding a second heterologous fusion protein comprising the second peptide or a segment thereof joined to a transcriptional activation protein transcriptional activation domain;

wherein binding of the first peptide or segment thereof and the second peptide or segment thereof reconstitutes a transcriptional activation

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protein; and

c) a luciferase gene activated under positive transcriptional control of the reconstituted transcriptional activation protein;

(ii) incubating [a] the test sample with the yeast cell under conditions suitable to detect the selected phenotype; and

(iii) detecting the interaction of the first peptide and the second peptide by determining the level of expression of the luciferase gene.

39. (Amended) The method of claim [37] 38, wherein the yeast cell is *Saccharomyces cerevisiae*.

43. (Amended) A method for determining whether a test sample interacts with a first or second peptide of a peptide binding pair, comprising:

(i) culturing at least one first yeast cell, wherein the first yeast cell comprises;

a) a nucleotide sequence encoding a first heterologous fusion protein comprising the first peptide or a segment thereof joined to a transcriptional activation protein DNA binding domain;

b) a nucleotide sequence encoding a second heterologous fusion protein comprising the second peptide or a segment thereof joined to a transcriptional activation protein transcriptional activation domain;

wherein the nucleotide sequence encoding the first heterologous fusion protein is present in an effective copy number of at least 5 copies per yeast cell and the nucleotide sequence encoding the second heterologous fusion protein is present at a copy number of 1 or 2 per yeast cell;

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and

wherein binding of the first peptide or segment thereof and the second peptide or segment thereof reconstitutes a transcriptional activation protein; and

c) a luciferase gene activated under positive transcriptional control of the reconstituted transcriptional activation protein;

(ii) culturing at least one second yeast cell, wherein the second yeast cell comprises;

a) a nucleotide sequence encoding a first heterologous fusion protein comprising the first peptide or a segment thereof joined to a transcriptional activation protein DNA binding domain;

b) a nucleotide sequence encoding a second heterologous fusion protein comprising the second peptide or a segment thereof joined to a transcriptional activation protein transcriptional activation domain;

wherein the nucleotide sequence encoding the second heterologous fusion protein is present in an effective copy number of at least 5 copies per yeast cell and the nucleotide sequence encoding the first heterologous fusion protein is present at a copy number of 1 or 2 per yeast cell;

and

wherein binding of the first peptide or segment thereof and the second peptide or segment thereof reconstitutes a transcriptional activation protein; and

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c) a luciferase gene activated under positive transcriptional control of the reconstituted transcriptional activation protein;

(iii) incubating a test sample with the first and second yeast cells under conditions suitable to detect luciferase activity;

(iv) detecting the [presence or absence of] luciferase activity produced by the first and second yeast cells; and

(v) comparing the detected luciferase activity of the first and second yeast cells, wherein [a change in the] lower luciferase activity in one of the yeast cells compared to the other yeast cell indicates that the test sample binds to the heterogeneous fusion protein encoded by the nucleotide sequence present at a copy number of 1 or 2 in that yeast cell exhibiting lower luciferase activity, thereby affecting the binding interaction of the peptide binding pair.

44. (Amended) The method of claim 43 wherein [the yeast cell] either or both of the first and second yeast cells further comprises at least one endogenous nucleotide sequence selected from the group consisting of a nucleotide sequence encoding the transcriptional activation protein DNA binding domain, and a nucleotide sequence encoding the transcriptional activation protein transcriptional activation domain wherein at least one of the endogenous nucleotide sequences is inactivated by reconstitution or deletion.

52. (Amended) The method of claim 43 wherein [the yeast cell] either or both of the first and second yeast cells comprises:

a) a nucleotide sequence encoding a first heterologous fusion protein

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comprising a first peptide of a peptide binding pair that bind through extracellular interaction in their natural environment, or a segment thereof, joined to a transcriptional activation protein DNA binding domain;

b) a nucleotide sequence encoding a second heterologous fusion protein comprising a second peptide of the binding pair, or a segment thereof, joined to a transcriptional activation protein transcriptional activation domain;

wherein binding of the first peptide or segment thereof and the second peptide or segment thereof reconstitutes a transcriptional activation protein; and

c) a luciferase gene activated under positive transcriptional control of the reconstituted transcriptional activation protein.

58. (Amended) A method of simultaneously detecting the interaction of two different peptide binding pairs in the presence of at least one test sample, wherein the first peptide binding pair comprises a first peptide and a second peptide, and wherein the second peptide binding pair comprises a third peptide and a fourth peptide, comprising:

(i) culturing at least one yeast cell, wherein the yeast cell comprises;

a) a nucleotide sequence encoding a first heterologous fusion protein comprising the first peptide or a segment thereof joined to a DNA binding domain of a first transcriptional activation protein;

b) a nucleotide sequence encoding a second heterologous fusion protein comprising the second peptide or segment thereof joined to a transcriptional activation domain of the first transcriptional

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activation protein;

c) a nucleotide sequence encoding a third heterologous fusion protein comprising the third peptide or segment thereof joined to a DNA binding domain of a second transcriptional activation protein;

d) a nucleotide sequence encoding a fourth heterologous fusion protein comprising the fourth peptide or a segment thereof joined to a transcriptional activation domain of the second transcriptional activation protein;

wherein binding of the first peptide or segment thereof and the second peptide or segment thereof reconstitutes the first transcriptional activation protein, and binding of the third peptide or segment thereof and the fourth peptide or segment thereof reconstitutes the second transcriptional activation protein;

[c)] e) a first luciferase gene activated under positive transcriptional control of the first reconstituted transcriptional activation protein;

[d)] f) a second luciferase gene activated under positive transcriptional control of the second reconstituted transcriptional activation protein; and

(ii) incubating the at least one test sample with the yeast cell under conditions suitable to detect luciferase activity; and

(iii) detecting the interaction of the first peptide and the second peptide by determining the level of expression of the first [and second luciferase genes] luciferase gene and detecting the interaction of the third peptide and the fourth peptide by determining the level of expression of the

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second luciferase gene.

64. (Amended) The method of claim [62] 63 wherein the peptide is a growth factor selected from the group consisting of epidermal growth factor, nerve growth factor, leukemia inhibitory factor, fibroblast growth factor, platelet-derived growth factor, vascular endothelial growth factor, tumor necrosis factor, oncostatin M, ciliary neurotrophic factor, erythropoietin, steel factor, placental lactogen, and TGF.

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08/259,609

PATENT COOPERATION TREATY

#11

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

To:
American Cyanamid Company
Attn. WEBSTER, Darryl L.
One Cyanamid Plaza
Wayne, New Jersey 07470
UNITED STATES OF AMERICA

Applicant's or agent's file reference ACY 32,352	Date of mailing *(day/month/year)* 13/10/95 **FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/US95/06895	International filing date *(day/month/year)* 31/05/95
Applicant AMERICAN CYANAMID CO.	

1. [X] The applicant is hereby notified that the international search report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the international search report; however, for more details, see the notes on the accompanying sheet.

Where? To the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Fascimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2; the applicant is notified that:

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicants's request to forward the texts of both the protest and the decision thereon to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer A. Siepkes

Form PCT/ISA/220 (July 1992)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
ACY 32,352		
International application No.	International filing date (day/month/year)	(Earliest) Priority Date (day/month/year)
PCT/US 95/06895	31/05/95	14/06/94
Applicant		
AMERICAN CYANAMID CO.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [ ] **Certain claims were found unsearchable** (see Box I).

2. [ ] **Unity of invention is lacking** (see Box II).

3. [ ] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
   - [ ] filed with the international application.
   - [ ] furnished by the applicant separately from the international application,
     - [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
   - [ ] Transcribed by this Authority

4. With regard to the **title,**
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:

   Figure No. 3
   - [ ] as suggested by the applicant.
   - [X] because the applicant failed to suggest a figure.
   - [ ] because this figure better characterizes the invention.
   - [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 95/06895

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/00 C12N1/19 C12N15/18 C12Q1/68 C12N15/62

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12Q C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 88, November 1991 WASHINGTON US, pages 9578-9582, C-T.CHIEN ET AL. 'The two-hybrid system: A method to identify and clone genes for proteins that interact with a protein of interest' cited in the application see page 9581 ---	1,2,4,6, 7,9,11, 15-20, 31-40, 42-48
X	SCIENCE, vol. 257, 31 July 1992 LANCASTER, PA US, pages 680-682, X.YANG ET AL. 'A protein kinase substrate identified by the two-hybrid system' cited in the application see the whole document --- -/--	1,2,4,6, 7,9,11, 15-20, 31-40, 42-48

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
20 September 1995	13. 10. 95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Cupido, M

Form PCT/ISA/210 (second sheet) (July 1992)

International Application No
PCT/US 95/06895

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X.	US,A,5 283 173 (S.FIELDS AND O-K.SONG) 1 February 1994 see the whole document	1,2,4,6, 7,9,11, 15-20, 31-40, 42-48
X	WO,A,94 09133 (THE GENERAL HOSPITAL CORPORATION) 28 April 1994 see page 1, line 14 - page 5, line 2	1,8-15, 18-20, 31-40, 42-48
X	CELL, vol. 74, 16 July 1993 NA US, pages 205-214, A.VOJTEK ET AL. 'Mammalian Ras interacts directly with the serine/threonine kinase Raf' see page 206, left column, paragraph 3	1,2,4, 6-15, 18-20, 31-40, 42-48
E	WO,A,95 18380 (THE SALK INSTITUTE FOR BIOLOGICAL STUDIES) 6 July 1995 see claims 1-19	1-7,9, 10, 12-20, 31-40, 42-48
E	WO,A,95 19988 (ICOS CORPORATION) 27 July 1995 see claims 1-9,18	1,2,4,6, 7,9,11, 15-20, 31-40, 42-48

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 95/06895

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
US-A-5283173	01-02-94	NONE		
WO-A-9409133	28-04-94	US-A-	5322801	21-06-94
		AU-B-	5325594	09-05-94
		EP-A-	0665884	09-08-95
WO-A-9518380	06-07-95	AU-B-	1436695	17-07-95
WO-A-9519988	27-07-95	NONE		